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"The Rapid Evolution of Complex Systems

from Simple Beginnings,"

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Response to a number of earlier comments will occur spontaneously in this presentation. I want to emphasize, however, that experiments indicate that proteinoids would not have had to evolve to information content; they had this quality from the outset [1]. This information is expressed in selective interactions with enzyme substrates [2], selective interactions with polynucleotides [3], and in selective assembly into supramolecular systems.

Amino acids represent an early stage in organic evolution; methods for their geological production have been much studied [4]. Our interest here centers on what can happen after the amino acids are polymerized. At this point, we need to consider a lingering difficulty in concept.

Carothers, who gave us the nylons through thermal polymerization of amino acids (if we use the term loosely), proposed that α-amino acids could not be polymerized thermally [5]. When we examine this concept, distinction between structural types of amino acid becomes crucial.

In essence, Carothers polymerized ω-amino acids

$$H_3^+N(CH_2)_nCOO^- \quad n = 4\text{-}6$$

The amino acids from protein are, however, α-amino acids:

$$H_3^+NCHRCOO^-$$

Carothers' inference is correct for most (the "neutral") amino acids. What has been learned beyond Carothers' inference is that amino acid mixtures containing basic amino acids,

e.g. $H_3^+N(CH_2)_4CHNH_3^+COO^-$ lysine

or acidic amino acids

$^-OOCCH_2CHNH_3^+COO^-$ aspartic acid

$^-OOC(CH_2)_2CHNH_3^+COO^-$ glutamic acid

can be copolymerized by heat, to include all of the proteinous amino acids. When such mixtures are heated, the polymers which are produced more closely resemble the proteins than the polymers Carothers and his students produced in the effort to simulate the protein of silk.

Our purpose in using a mixture of as many as eighteen kinds of amino acid is based on recognition of the potentiality for isomerism which is unique to protein or protein-like molecules [6]. The vast numbers possible during evolution provide a potential matrix for arrays of differing specificities, such as would be needed for enzymes permitting the evolution of metabolism. Moreover, geologically relevant experiments show that amino acids are typically produced in sets, or families [7].

Since polymerization is rapid, the kind of complexity represented by varieties of heteropolyamino acids is the kind, I have been told, that has been of interest to physicists. The relevance to fundamental physical thought is increased by the rapidity and ease with which the polymers assemble into complex microsystems. This kind of study is constructionistic in contrast to much of biology, which proceeds by disassembling complex contemporary systems. I believe that only by assembling small synthetic components to macromolecules and thence to a kind of cell [8] can we hope to appreciate how easily complexity arises from simple beginnings by simple processes.

When the conditions are geologically relevant, as those in our studies are [4], the reactions constitute a model of preDarwinian evolution.

Figure 1 demonstrates the kind of result known to Carothers (on the left). On the right is the clean proteinoid (protein-like polymer) obtained by including sufficient proportions of aspartic acid and glutamic acid, heating to 170° for 6 hours, and purifying the polymer from a light pigment.

The amino acids were heated in an initially dry state to overcome the energetic barrier which exists in aqueous solution [9]. The temperatures used are sufficient both to distill water from aqueous solutions and to polymerize the dried residue [4]. The requirement of a hypohydrous environment is, in my opinion, also met by the use of montmorillonite, with adenylates. Reactions facilitated at such surfaces are not occurring in dilute aqueous solution.

While the objective of employing eighteen types of amino acid was pointed mainly toward later evolutionary development [6], the thermal coupling of two to eighteen types of amino acid has been shown to yield polymers with much internal order. The various kinds of evidence, all of which require this inference, are shown in Table 1. A striking instance is seen in Fig. 2, kindly provided by Professor Klaus Dose [10].

We visualize that the ordering is due to selective interaction of amino acids, also shown in other systems [11,12], and especially to thermal rearrangement to increasingly thermodynamically stable sequences. The result is almost at the other extreme from the <u>a priori</u> theoretical calculations based on an assumption of randomness. That assumption is unjustified for this coupling process, as the many results show. The significance to the primordial sequence is that the first ordered proteins did not require prior nucleic acids. This experimentally derived fact resolves a number of chicken-egg dilemmas.

One of these dilemmas is how enzymes came into existence when no enzymes existed to make them. The ordering of amino acids in proteins to produce catalytically active polymers is better understood in association with an explanation of that order.

Internal compositional ordering is observed also in the copolymerization of aminoacyl adenylates, which has been studied [13], in an approach to understanding the origin of the genetic code [3].

Table 2 presents a summary of the enzymelike activities which have been identified in various thermal proteinoids by various laboratories. These include in a number of instances pH-activity curves of the usual type, Michaelis-Menten kinetics, heat inactivation in aqueous solution, specificity of interaction between proteinoid and substrate, enhancement of five types of reaction, and a basis for the origin of metabolic pathways [2]. Typically, each kind of proteinoid has its array of (weak) enzymelike activities, and the variety of proteinoids permits an array of arrays.

Comparison of the tests with those of contemporary enzyme proteins has shown generally an absence of activities in the evolved protein, except for its specialized power. This has supported the inference that macromolecular evolution could not have begun with any highly specialized evolved protein; a heteropolyamino acid possessing a number of enzyme-like activities in small degree was what was needed.

The way in which water entered and left the evolutionary development also requires precise understanding. We know that contact of thermal polyamino acids (proteinoids) with water produces, in a maximally simple operation, vast numbers of microsystems (Fig. 3) with many of the properties of contemporary cells. These properties are listed in Table 3.

The microsystems produced are uniform in size, numerous (10^7-10^9 per gram), dynamic (they shrink in response to hypertonic solution and swell in hypotonic solution), they can be made to stain Gram-positive or Gram-negative, they have the size, shape, and pattern of association of some cocci, and their stability overlaps the range of stability of contemporary cells.

The fact that the units are stable has permitted embedding and microtoming them for electron microscopy. An electron micrograph is seen in Fig. 4.

In Fig. 5 is presented, through the optical microscope, a kind of replication [1]. It is an explanation of how cells first came to replicate. With this observation, we can construct answers of how a primitive cell [8] could have arisen when no cells existed to produce them, how they contained protoenzymes when no enzymes existed to make them, and how informational macromolecules arose from heated amino acids. With these phenomena organic evolution, as we know it, could have begun.

I would like to turn next to recently reported observations indicating that such microsystems possess an inherent tendency to communicate [14]. These results also illustrate the kind of phenomenon mentioned by Dr. Szent-Gyoergyi; they particularize how biological phenomena might have emerged from the action of structural constraints on random (Brownian) motion.

Figure 6 displays a time-lapse sequence of proteinoid microspheres approaching each other. One pair is joined, another becomes joined, and a third vacillates between junction and separation.

Figure 7 presents the evidence that these junctions are indeed new structures. Figure 8 indicates that the junction permits the transfer from one microsphere to another of smaller particles, endoparticles. Since the endoparticles are composed of proteinoid, and proteinoid contains information as earlier defined, this is a model of the transfer of information.

Although Brownian motion may be random, the nonrandom deployment of the portals in the total structure results in ordered behavior.

The possibility that these systems involve Brownian motion renders them appropriate for physical study. In another sense, all aspects of the model described are amenable to physical study. A basic premise that has permitted such phenomenological experiments is that biology is ultimately redicible to physics. The opposing proposition results in no experiments and no new knowledge, and is thus a self-fulfilling premise.

Acknowledgment

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Fig. 1. On left, tube containing mixture of amino acids heated to above the boiling point of water; product is dark and tarry. On right, granular polymer prepared by heating a mixture of amino acids containing sufficient proportions of aspartic acid and glutamic acid; product is light or white depending upon details of preparation.

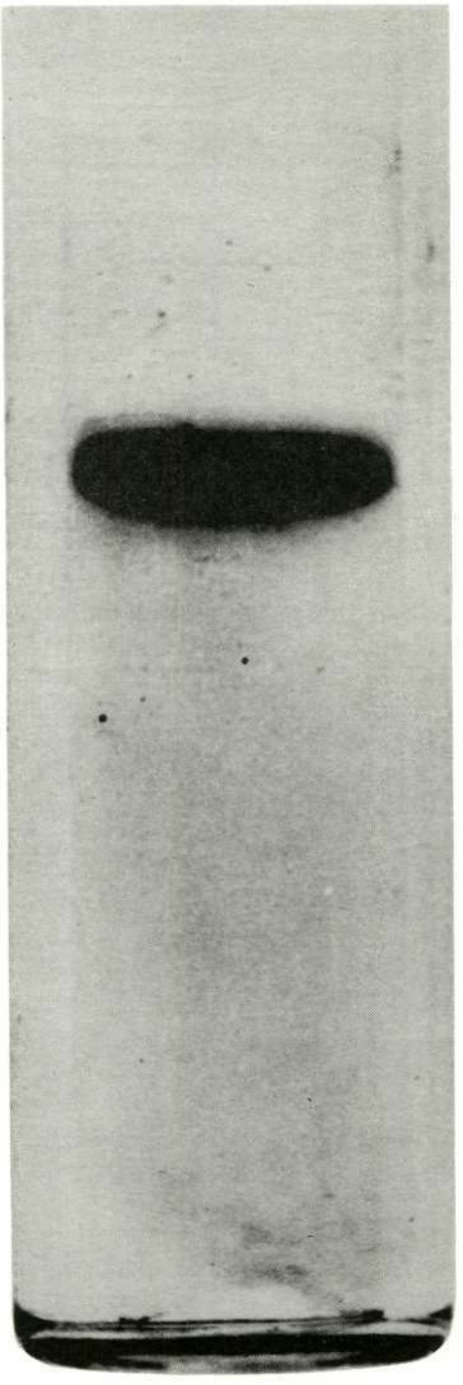

Fig. 2. Acrylamide gel electrophoresis of hemoproteinoid 83a at pH 8.6 [10]. Colored by Amidoschwarz 10B. The preparation appears to be homogeneous also by the use of electrophoresis at pH 4.5 and gel filtration.

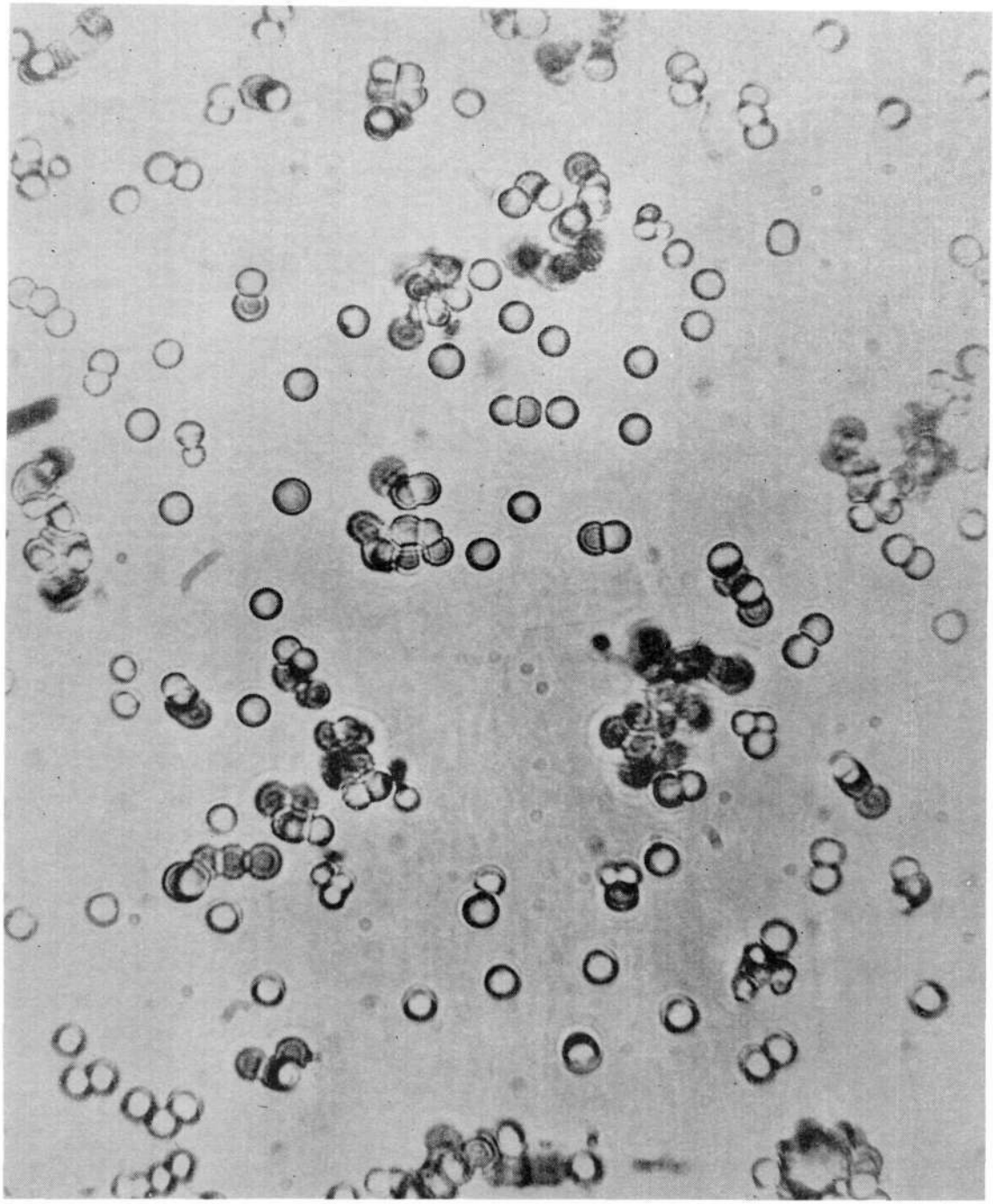

Fig. 3. Proteinoid microspheres produced by heating proteinoid in aqueous solution and cooling. They are typically uniform in the range of 1-2 μm in diameter.

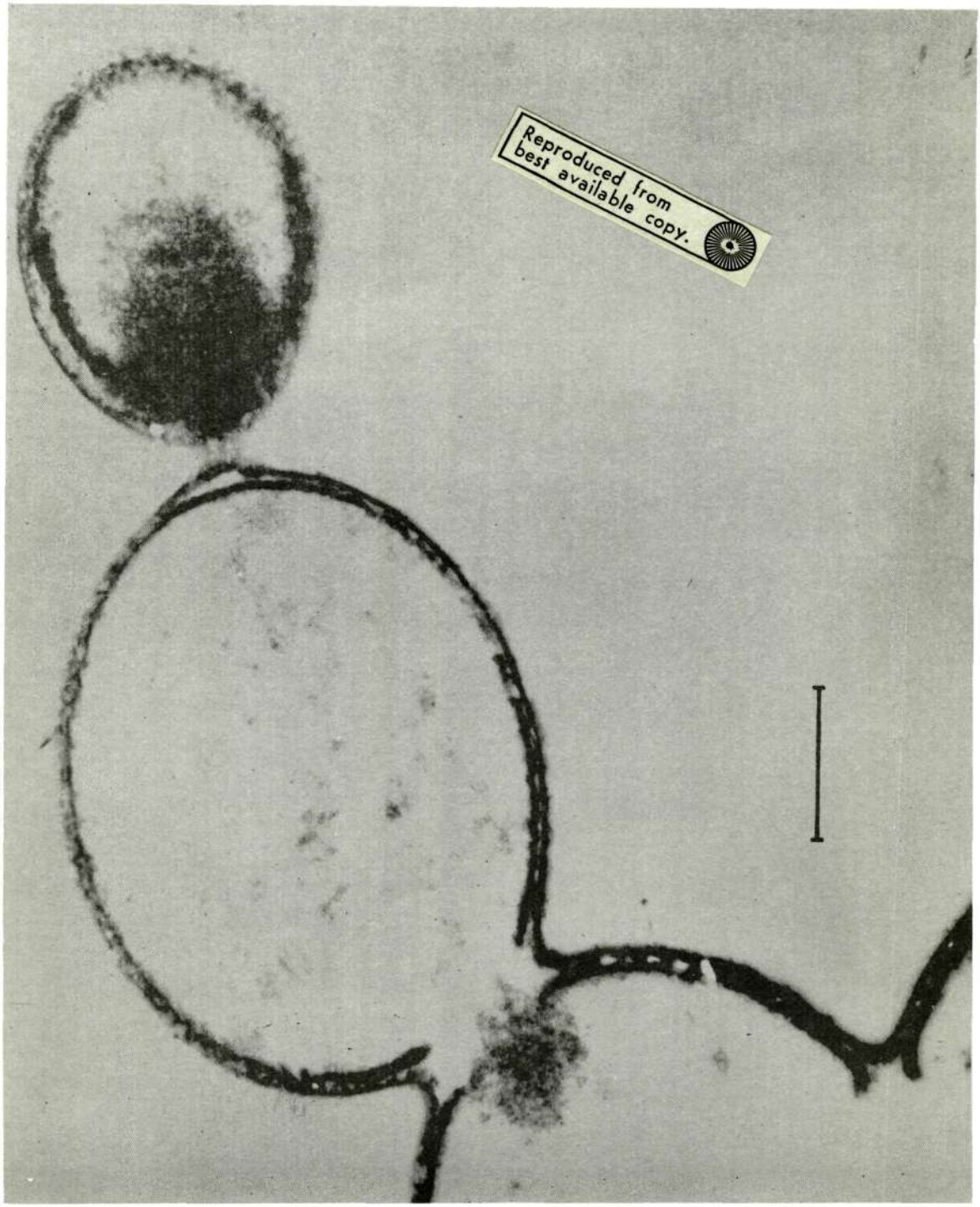

Fig. 4. Electron micrograph of proteinoid microsphere subjected to minor elevation of pH. Stained with osmium tetroxide and embedded in methacrylate. Note double layers.

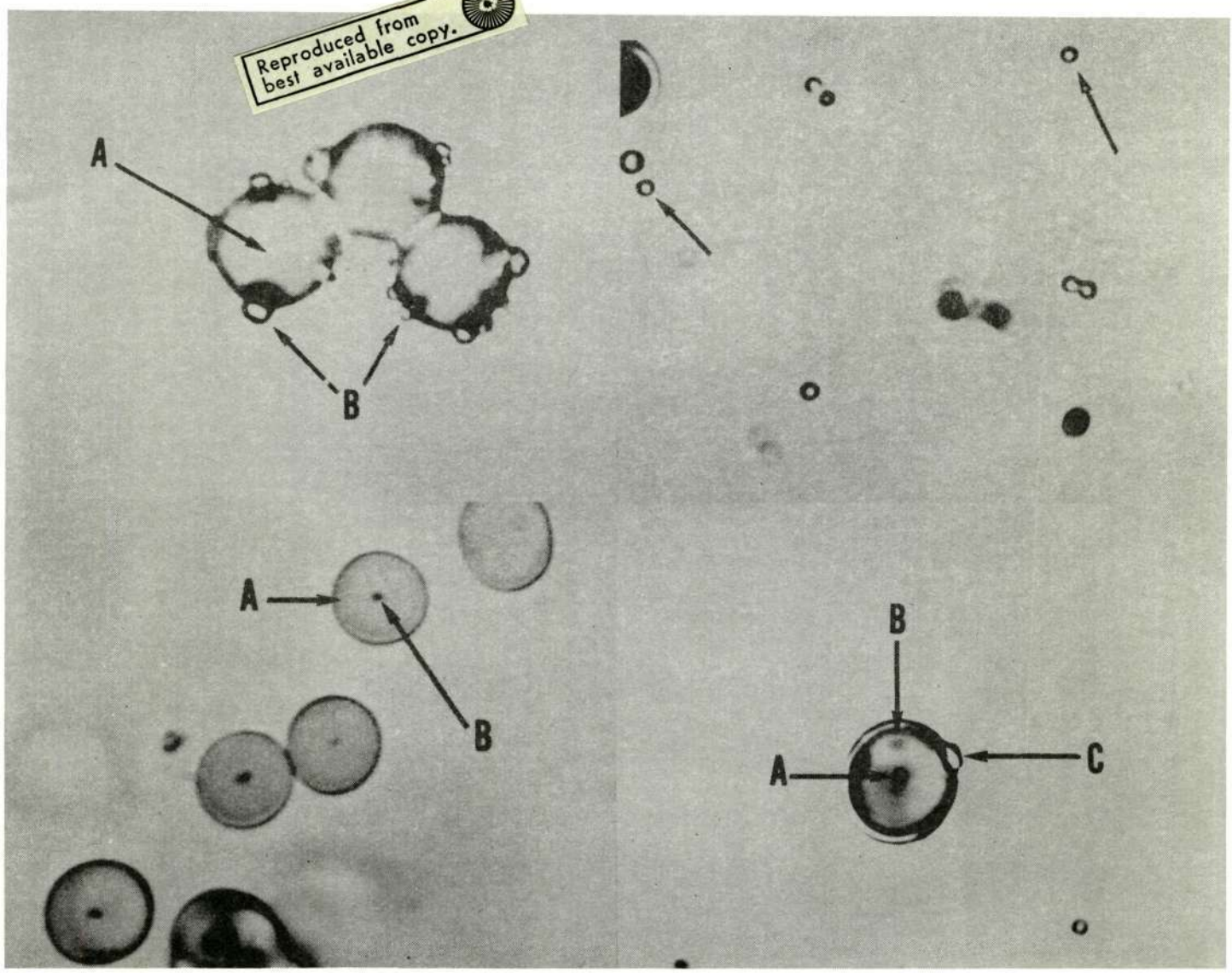

Fig. 5. Replication of proteinoid microspheres. a) Budded microspheres produced by standing. b) Buds liberated mechanically. c) Stained buds around which second generation microspheres have grown by accretion (temperature of solution saturated at 37° has dropped to 25°). d) Second generation bud (C) on second generation microsphere (B) accreted around first generation bud (A). Microspheres approximately 12-15 mμ in diameter.

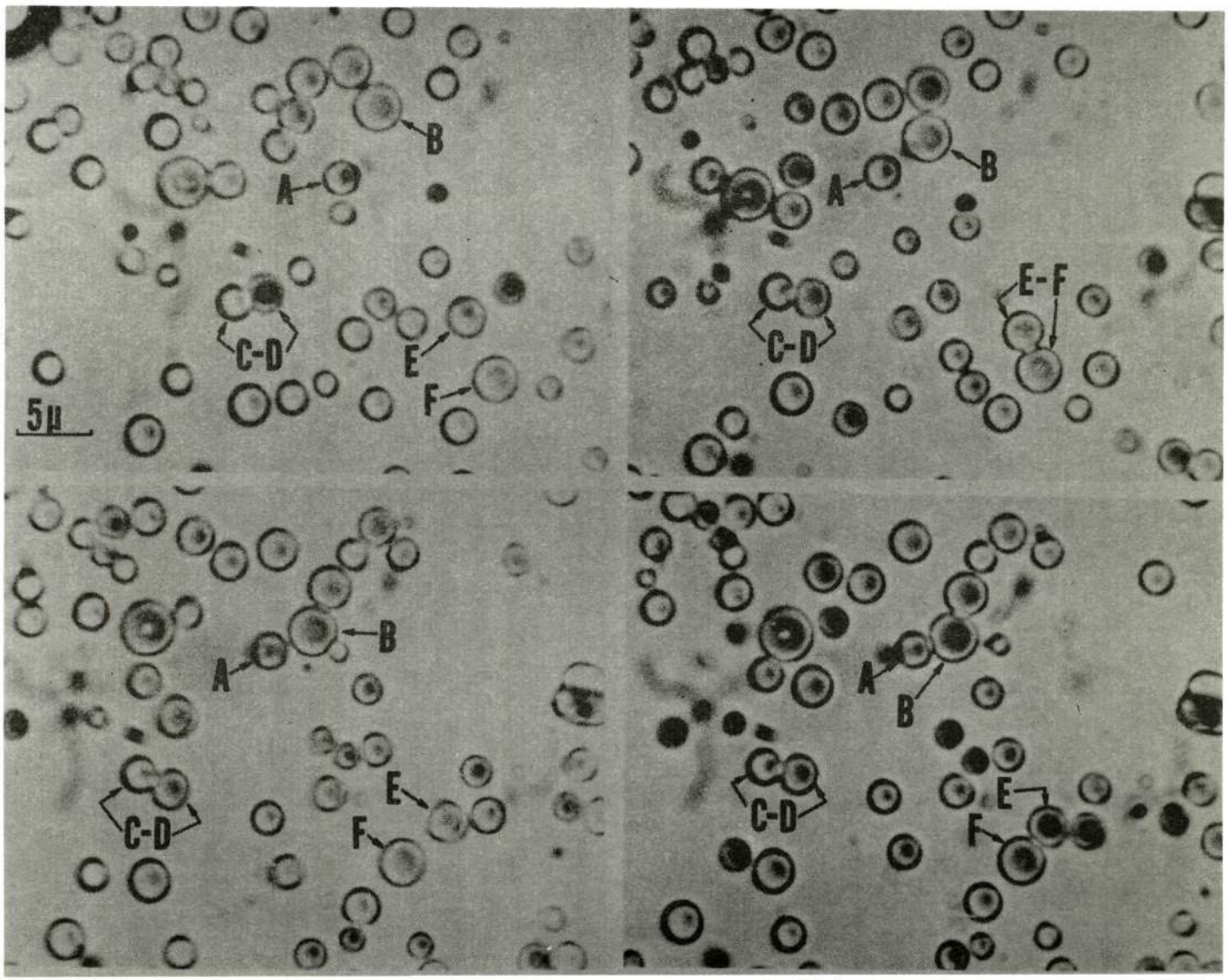

Fig. 6. Proteinoid microspheres forming junctions. Ten second time-lapse. A and B join and remain joined. C and D are joined throughout. E and F join, separate, join, and separate. This behavior is often seen. Junctions are visible.

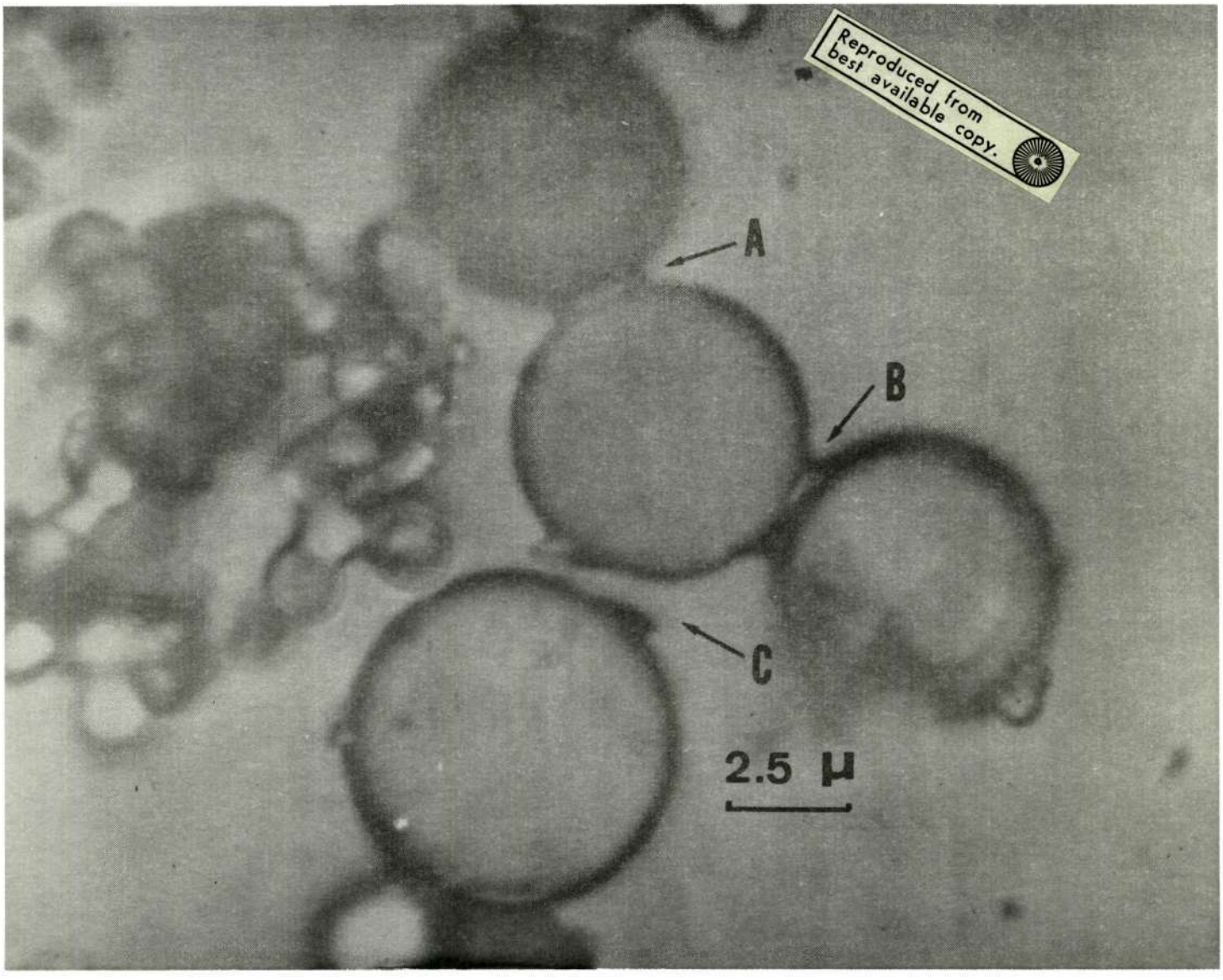

Fig. 7. Stained joined microspheres (Crystal Violet). A is intact, B is cracked, C has separated. Junctions are new structures formed from microsystems composed of preformed polymer.

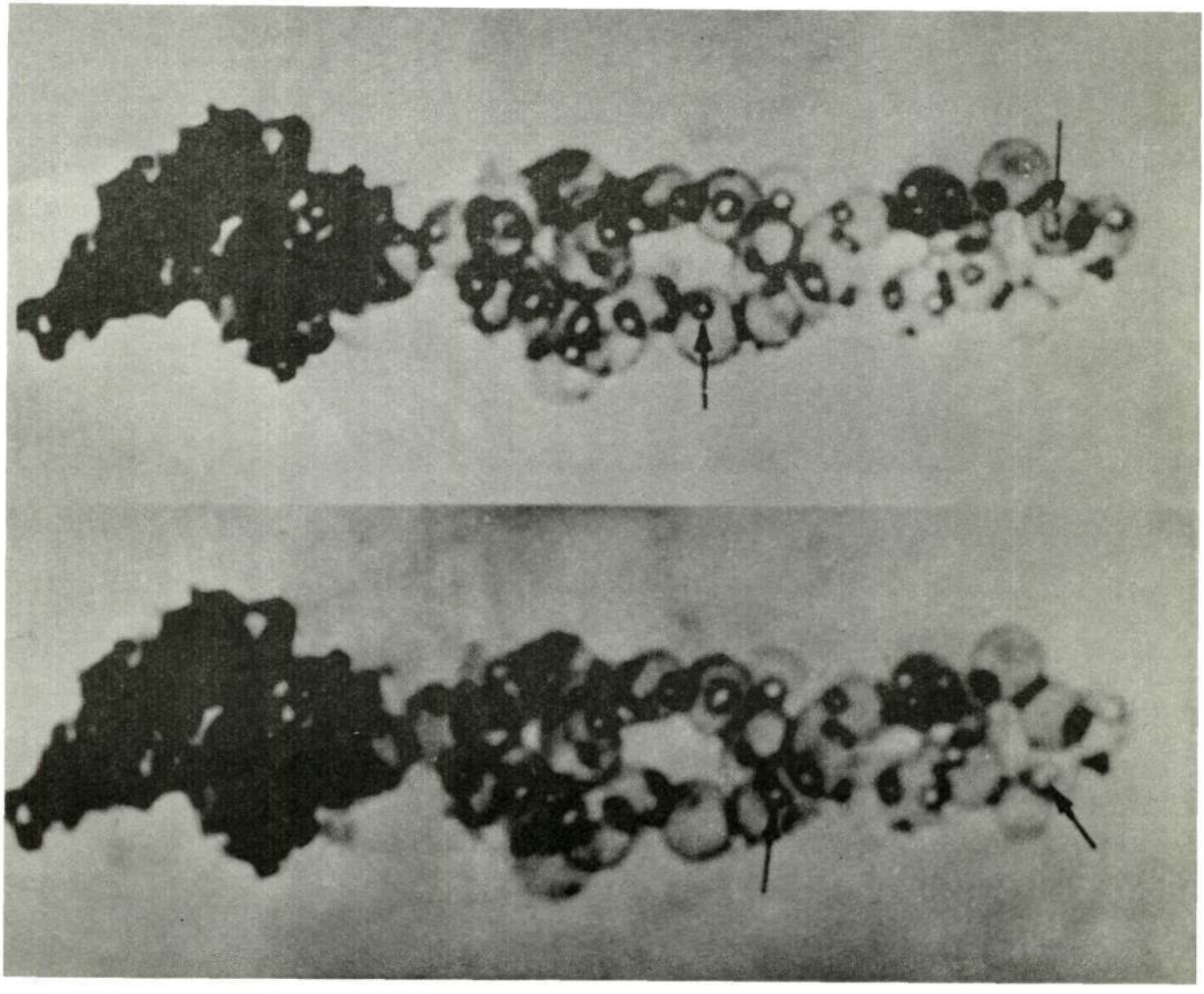

Fig. 8. Two time-lapse frames [14] showing transfer of endoparticles from one proteinoid microsphere to another through hollow junction.

Table 1

Self-ordering in the Thermal Condensation of

Amino Acids

Evidence	Date and Authors
Nonrandom sequences by disparity between N-terminal and total analyses in thermal polymers	1958: Fox and Harada
%s in reaction mixture ≠ in polymer	1960: Fox and Harada
Two peaks from proteinoids on electrophoresis	1960: Vestling
Limited heterogeneity on ultracentrifugation	1961: Vegotsky
Constant composition on repurification from water	1963: Fox, Harada, Woods, Windsor
Single band on gel electrophoresis for acidic proteinoidamide	1966: Fox and Nakashima
Nonrandom elution pattern from DEAE-cellulose	1967: Fox and Nakashima
Symmetrical peaks from DEAE-cellulose	
Almost uniform amino acid compositions in various fractions	
Stoichiometric amino acid compositions	
Uniform ultracentrifugal patterns of various fractions	
Almost uniform peptide maps in all fractions	
Single spots on high voltage electrophoresis of fractions	
Single species of "active site" proteinoids	1968: Usdin, Mitz, and Killos
Single band for gel electrophoresis of basic hemoproteinoid	1971: Dose and Zaki

Table 2

Catalytic Activities in Thermal Proteinoids

Reaction and Substrate	Remarks	Authors and Year
Hydrolysis		
p-Nitrophenyl acetate	Activity of proteinoid greater than of equivalent free histidine	Fox, Harada, and Rohlfing (1962)
p-Nitrophenyl acetate	Thermal polymers most active	Noguchi and Saito (1962)
p-Nitrophenyl acetate	Inhibition by organic phosphates; reversal	Usdin, Mitz, and Killos (1967)
p-Nitrophenyl acetate	General description	Rohlfing and Fox (1967)
p-Nitrophenyl acetate	Reactive site, and inactivation	Rohlfing and Fox (1967)
ATP	Through Zn salt	Fox and Joseph (1965)
p-Nitrophenyl phosphate	A second phosphate Hydrolysis	Oshima (1968)
Decarboxylation		
Glucuronic acid	From glucose, CO_2	Fox and Krampitz (1964)
Pyruvic acid	→ acetic acid + CO_2; Michaelis-Menten kinetics	Krampitz and Hardebeck (1966) Hardebeck, Krampitz, and Wulf (1968)
Oxaloacetic acid	Rapid, requires basic polymers	Rohlfing (1967)
Amination		
α-Ketoglutaric acid	Requires both Cu^{++} and proteinoid	Krampitz, Diehl, and Nakashima (1967)

Table 2 (cont'd)

Reaction and Substrate	Remarks	Authors and Year
Deamination		
Glutamic acid	Requires both Cu^{++} and proteinoids	Krampitz, Haas, and Baars-Kiehl (1968)
Oxidoreductions		
H_2O_2 (catalase reaction)	Activity of hemin lowered when incorporated into proteinoids	Dose and Zaki (1971)
H_2O_2 and hydrogen donors (guaiacol, hydroquinone, NADH, and others) (peroxidase reaction)	Activity of hemin increased up to 50 times in lysine-rich hemoproteinoids	Dose and Zaki (1971)

Table 3

Properties of Proteinoid Microparticles

Stability (to standing, centrifugation, sectioning)
Microscopic size
Variability in shape
Uniformity of size
Numerousness
Stainability
Producibility as gram-positive or gram-negative
Shrinking or swelling in atonic solutions
Structured boundary
Ultrastructure (electron microscope)
Selective passage of molecules through boundary
Assembled from catalytically active polymer
Patterns of association
Budding and fission
Growth by accretion
Ability to propagate through budding and growth by accretion
Ability to form junctions
Ability to communicate information